# SYNTHESIS OF ETHYL N-CARBOBENZOXYTYROSYL-S-BENZYLCYSTEINYLGLYCINATE

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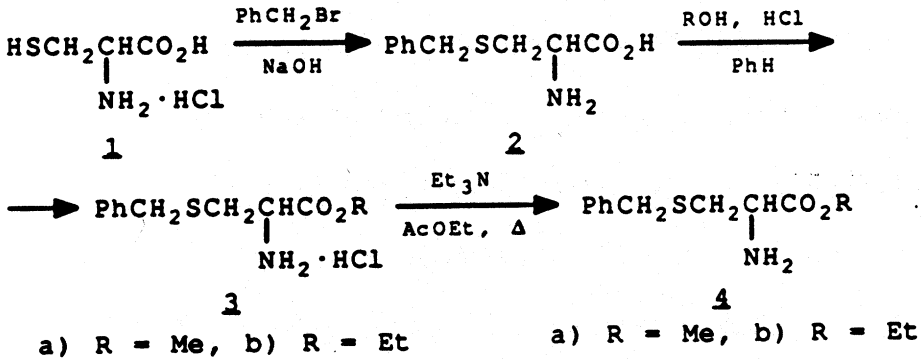
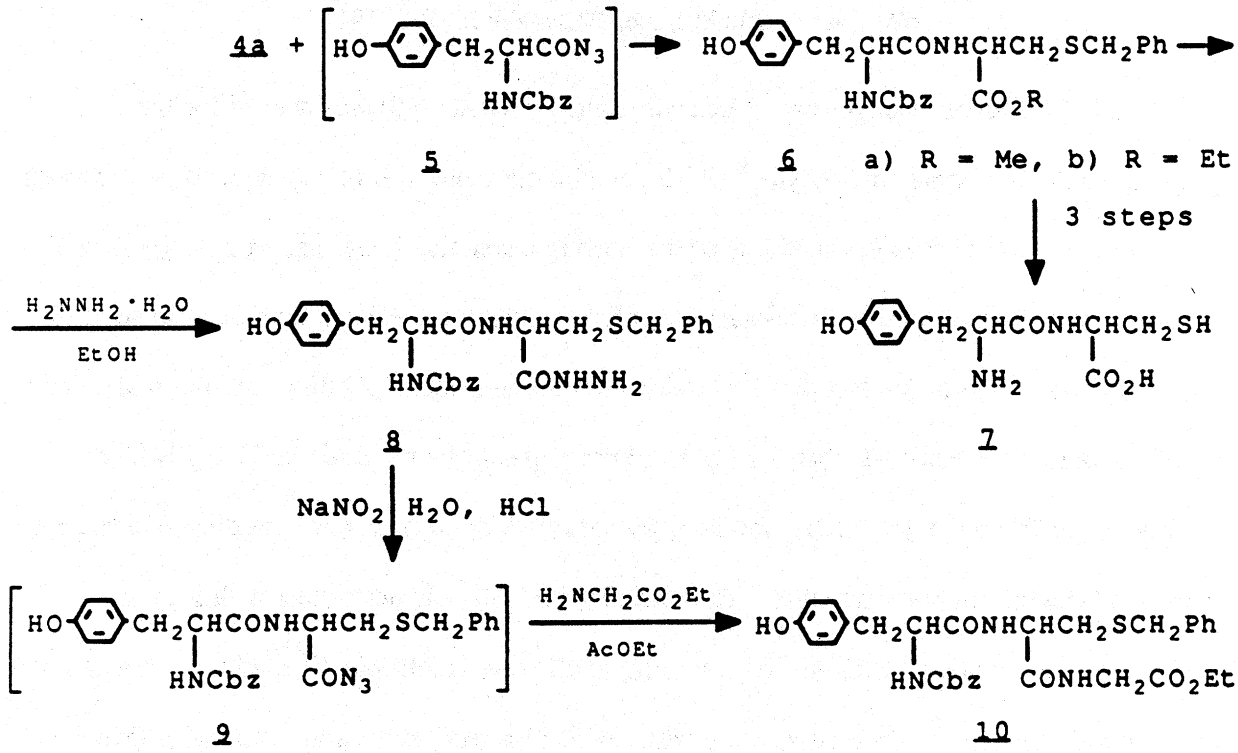
Ethyl N-carbobenzoxytyrosyl-S-benzylcysteinylglycinate (10) was needed for the preparation of heat stable enterotoxin,[1] which contains six molecules of cysteine. In that context it was essential to improve the synthesis of the starting materials, 2, 3a, 3b, 4a and 4b, since the original preparation of S-benzyl-L-cysteine (2, 85% yield) proved far too long and too complex.[2] Advantage was taken of the fact that L-cysteine does not undergo rapid dimerization in alkaline solutions and thus S-benzyl-L-cysteine (2) could conveniently be prepared in 84% yield by dissolving L-cysteine in 1N NaOH, followed by benzylation; all reactions including acidification were conducted at room temperature. The esterification of 2 was performed with HCl in methanol for preparation of the methyl ester (3a) and by the azeotropic distillation for preparation of the ethyl ester (3b).[3] Both esters were best purified by recrystallization from isopropanol instead of from ethanol. These salts were converted to the free bases 4a and 4b in better yields and purity by the use of triethylamine instead of aqueous sodium hydroxide.[4]



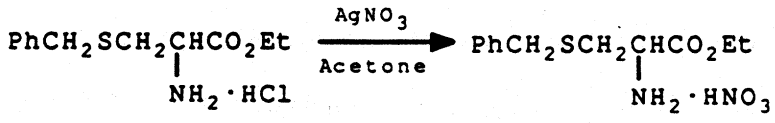
Coupling of 4a with N-carbobenzoxy-L-tyrosyl azide (5) produced 6a in 60% yield. Compound 5, stable only in inert solvents such as $Et_2O$ and EtOAc, has been prepared from its hydrazide[12] and it is known that acyl azides react easily with amino acid esters without racemization.[5] The structure of similarly prepared 6b was established by conversion to the known 7 by the procedure of Roberts and Du Vigneaud.[6] The methyl ester 6a was converted to its hydrazide and then to the azide (9) which was coupled in situ with ethyl glycine to yield 10.




The azide method for coupling has not been extensively utilized[7] but it appears to be very suitable for the syntheses of higher peptides,[8] and this has been again demonstrated in the present work. We also found that methyl esters of amino acids and peptides are more suitable than the ethyl esters for these syntheses, reacting faster and in better yields (6a).

An attempt to prepare the free base 4b from 3b by using silver nitrate in acetone gave unexpectedly a previously unknown nitrate salt (11). Apparently this is the only way to prepare the nitrate salt of 3b, which possesses unusual properties; although it is a salt, it is soluble in

ether, acetone and in aliphatic alcohols. To our knowledge, this is the first report of a nitrate salt of an amino acid.

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## EXPERIMENTAL SECTION

L-Cysteine hydrochloride dihyhrate, hydrazine monohydrate, and other common chemicals were obtained from commercial sources. Optical rotations were determined on a Perkin-Elmer 141 polarimeter,[9] using a standard 10 cm cell, volume 5 ml. IR spectra were determined on a Nicolet 740 FTIR spectrophotometer at 2 $cm^{-1}$ resolution.

S-Benzyl-L-cysteine (2).—To a stirred, clear solution of cysteine hydrochloride dihydrate, (17.56 g, 0.1 mole), in 240 ml 1N NaOH in a 500 ml Erlenmeyer flask, 0.1 mole of benzyl bromide, or chloride, was added dropwise. After 1 hr the addition was complete and stirring was continued for an additional 3-4 hr; the reaction mixture stored at room temperature for 48 hr. During that time some of the product precipitated. Precipitation was completed by acidification with concentrated HCl to about pH 3. The voluminous precipitate was collected, washed with 200 ml water and dried at 56°/0.1 mm Hg to yield 17.65 g (84%) of 2. This material was dissolved in 1400 ml boiling water (79-80 ml/g), the solution was filtered and stored at room temperature for about 48 hr. The needle-like crystalline product that formed was collected, washed twice with 100 ml water, then with 100 ml acetone, and dried at 56°/0.1 mm Hg, to give 12.77 g (60% yield) of pure, final product (2), mp. 218-220°; $[\alpha]_D^{25°} = +26.50°$ (c 1, 1 N NaOH), lit.[2] mp. 216-218°, $[\alpha]_D^{26.5°} = +23.5°$, 58% yield.

IR (KBr): 3159, 3030, 2946, 1620, 1587, 1562, 1495, 1453, 1394, 1318, 1279, 1156, 1071, 847, 767 and 695 $cm^{-1}$. The spectrum is identical with that reported by Keller.[10]

S-Benzyl-L-cysteine Methyl Ester Hydrochloride (3a).—A mixture of S-benzyl-L-cysteine (2) (21.17 g, 0.10 mole) and 300 ml of methanol previously saturated with hydrogen chloride, placed in a 500 ml flask equipped with a stirrer and a condenser and immersed in a silicone bath at about 75°, was stirred and refluxed for 3 hr; then the methanol was distilled off under reduced pressure and the residue dried at 56°/0.1 mm Hg. A nearly quantitative yield (25.75 g) of the ester hydrochloride was obtained; this was recrystallized from isopropanol (6 ml/g) to yield 17.2 g (66%) of the pure product, mp. 152-153°, $[\alpha]_D^{25°}$ = -14.5° (c 3, $H_2O$), lit.[11] mp. 151-152°. IR (KBr): 3112, 2673, 2570, 1745, 1239, 1103, 1039 and 704 $cm^{-1}$.

Anal. Calcd. for $C_{11}H_{16}ClNO_2S$: C, 50.47; H, 6.16; Cl, 13.54; N, 5.34; S, 12.24

Found: C, 50.18; H, 5.88; Cl, 13.73; N, 5.15; S, 12.42

S-Benzyl-L-cysteine Ethyl Ester Hydrochloride (3b).—This compound was prepared by using the azeotropic method of esterification.[3] S-Benzyl-L-cysteine (2) (21.13 g, 0.1 mole), 300 ml 95% ethanol, 35 ml concentrated hydrochloric acid and 300 ml benzene were placed into a 1000 ml three neck flask equipped with a stirrer and a condenser. The mixture was stirred and heated (mantle) until 800-850 ml of the azeotropic distillate was collected. During the course of distillation, an additional 300 ml of benzene and 100 ml of ethanol were added. Initially, the azeotropic mixture distilled at 65°, then the temperature gradually rose to 89°. At that point the mixture was refluxed for about 30 min amd then the alcohol was distilled off under reduced pressure. The residue which solidified on cooling to room temperature was dried at 56°/0.1 mm Hg, whereupon 26.80 g (98%) of 3b was obtained. The product was reasonably pure (96-98%) and is suitable for use, mp 153-156°. After recrystallization (87%) from isopropanol (7 ml/g) it had mp. 159-160°, $[\alpha]_D^{25°}$ = -24.60° (c 1, EtOH), lit.[4] mp. 156-157°. IR (KBr): 3145, 2867, 2813, 2751, 2673, 2639, 1742, 1495, 1421, 1382, 1227, 1197, 1099, 1045 and 855 $cm^{-1}$.

Anal. Calcd. for $C_{12}H_{18}ClNO_2S$: C, 52.25; H, 6.57; Cl, 12.85; N, 5.07; S, 11.62

Found: C, 51.89; H, 6.72; Cl, 12.68; N, 5.20; S, 11.45

S-Benzyl-L-cysteine Methyl Ester (4a).—S-Benzyl-L-cysteine methyl ester hydrochloride (3a) (26.2 g, 0.1 mole), 45 ml (0.3 mole) triethylamine and 250 ml ethyl acetate were placed into a 500 ml reaction flask equipped with a stirrer and a condenser and heated on a silicone bath for 30 min at about 75°. The reaction mixture was then filtered by suction and the filtrate concentrated under reduced pressure under a slow stream of nitrogen. A nearly theoretical yield (21.90 g) of yellowish, oil-like material was obtained. It contained some solid particles which were removed by filtration, using a fine porosity sintered glass funnel, yielding 21.55 g (96%) of the final product, $[\alpha]_D^{25°}$ = + 14.85° (c 1, $CH_3OH$); $n_D^{25°}$ 1.2858; $d_{25}$ 1.1100.

IR (film): 3380, 3029, 1736, 1243, 1185, 1028 and 704 $cm^{-1}$.

Anal. Calcd. for $C_{11}H_{15}NO_2S$: C, 58.63; H, 6.71; N, 6.20; 5, 14.23

Found: C, 58.40; H, 6.65; N, 5.95; S, 13.98

S-Benzyl-L-cysteine Ethyl Ester (4b).—S-Benzyl-L-cysteine ethyl ester hydrochloride (3b) (20.0 g, 0.0725 mole) was converted to the free base using the same procedure as given above for the preparation of 4a. A yellowish, oil-like material was also obtained, $[\alpha]_D^{25°}$ = + 15.60° (c 1, $CH_3OH$), $n_D^{22}$ 1.5365; $d_{22}$ 1.1120. The IR spectrum (film) of 4b is almost identical with that of 4a, except for two weak bands which are evident at 2982 and 2933 $cm^{-1}$. Both free esters show a tendency to solidify after standing at room temperature under nitrogen for about two weeks. Attempted distillation of either ester, even at a reduced pressure of about 0.01 mm Hg, resulted in decomposition.

Anal. Calcd. for $C_{12}H_{17}NO_2S$: C, 60.22; H, 7.16; N, 5.84; S, 13.39

Found: C, 59.86; H, 7.24; N, 5.75; S, 13.17

N-Carbobenzoxytyrosyl-S-Benzylcysteine Methyl Ester (6a).—N-Carbobenzoxy-L-tyrosylhydrazide[12] (8.65 g, 0.026 mole), 210 ml of 0.6 N HCl and 64 ml acetic acid were placed into a 500 ml Erlenmeyer flask; the mixture was stirred for a few min and the clear solution cooled to about 0°. To this was added dropwise a solution of 1.85 g of sodium nitrite in 18 ml water. The addition was completed in about 20 min. Stirring and cooling was continued for an additional 30 min;

then the reaction mixture was kept at room temperature for about 2 hr and extracted with ethyl acetate (3 x 100 ml). To the extract was added 6.42 g (0.0284 mole) of S-benzyl-L-cysteine methyl ester (4a), and the solution was stored at room temperature overnight. Then the solvent was distilled off at room temperature under reduced pressure under a slow stream of nitrogen. The residue was dried at 56°/0.2 mm Hg, and gave 9.24 g (68%) of the product (6a), mp. 160-162°; $[\alpha]_D^{25°}$ = - 41.0° (c 1, $CH_3OH$); - 36.2° (c 1, DMF). After recrystallization from methanol (25 ml/g), 8.30 g (61% yield) of pure product was obtained, mp. 170-170.5°; $[\alpha]_D^{25°}$ = - 34.3° (c 1, DMF).

IR (KBr): 3301, 1733, 1699, 1649, 1541, 1516, 1373, 1267, 1216, 1047 and 695 $cm^{-1}$.

Anal. Calcd. for $C_{28}H_{30}N_2O_6S$: C, 64.34; H, 5.78; N, 5.35; S, 6.13

Found: C, 64.09; H, 5.72; N, 5.18; S, 6.32

N-Carbobenzoxytyrosyl-S-Benzylcysteine Ethyl Ester (6b).—N-Carbobenzoxy-L-tyrosylhydrazide[12] (16.46 g, 0.05 mole), 420 ml of 0.6 N HCl and 130 ml acetic acid were placed into a 1000 ml Erlenmeyer flask, the mixture was stirred and cooled as above, and 4.0 g of sodium nitrite in 40 ml water was added and reacted as above. The reaction mixture was extracted with ethyl acetate (3 x 200 ml) and to the extract was added 13.15 g (0.553 mole) of S-benzyl-L-cysteine ethyl ester (4b). The solution was kept at room temperature overnight, the solvent distilled off as above, the residue dried at 56°/0.2 mm Hg and recrystallized from methanol (30 ml/g), whereupon 16.0 g (60%) of 6b was obtained, mp. 163-165°; $[\alpha]_D^{25°}$ = -37.2° (c 1, DMF), lit.[4] mp. 142-145°; lit.[6] mp. 168.5-169°.

IR (KBr): 3362, 3351, 3301, 1733, 1699, 1649, 1540, 1516, 1372, 1267, 1216, 1047, and 695 $cm^{-1}$.

Anal. Calcd. for $C_{29}H_{32}N_2O_6S$: C, 64.90; H, 6.01; N, 5.21; S, 5.77

Found: C, 64.55; H, 6.24; N, 5.37; S, 5.78

L-Tyrosyl-L-cysteine (7).—This compound was prepared to confirm the structure of 6a and 6b. The ethyl ester (6b) (6.0 g, 0.011 mole) was converted to 7 in 70% yield, mp. > 300°, by the

procedure of Harington and Pitt Rivers.[4] $[\alpha]_D^{25°}$ = + 22.9° (c 1, 1 N HCl); lit.[4] yield 66%, mp. > 300°; $[\alpha]_D^{23°}$ = + 22.6° (c 1, 1 N HCl).

IR (KBr): 3301, 3296, 3269, 2885, 2575, 1685, 1645, 1532, 1514, 1453, 1215, 1048, 664 and 548 $cm^{-1}$.

Anal. Calcd. for $C_{12}H_{16}N_2O_4S$: C, 50.69; H, 5.67; N, 9.84; S, 11.27

Found: C, 50.55; H, 5.80; N, 9.80; S, 11.14

N-Carbobenzoxytyrosyl-S-Benzylcysteinylhydrazide (8).—A mixture of 17.25 g (0.033 mole) of 6a and 500 ml methanol in a 1000 ml reaction flask equipped with a stirrer and condenser was stirred and heated at about 60° in a silicone bath for 15 min until a clear solution was obtained. To this was added 6.60 g (0.132 mole) of hydrazine hydrate (100%) and heating and stirring were continued for an additional 45 min. The solid that formed after storage at room temperature for about 40 hr was collected, washed with 100 ml methanol, and dried at 56°/0.2 mm Hg to yield 14.6 g (85%) of a light yellowish product, mp. 212-215°, $[\alpha]_D^{25°}$ = -17.6° (c 1, DMF). This material was insoluble in ether, and benzene, very slightly soluble in ethyl and methyl alcohols (about 0.17 g/100 ml). It was recrystallized from acetic acid, 10 ml/g; however, crystallization was very slow and inefficient. After standing for 72 hr at room temperature only a 50% yield of impure 8 was obtained, mp. 226-229°. Since the starting material (6a) is easily soluble in boiling alcohols, purification of the product was accomplished by suspending it in 500 ml methanol, boiling for 5 min, filtering hot, and drying the residue as above. The final product, 14.10 g (82%), was quite pure, mp. 231-231.5°; $[\alpha]_D^{25}$ = -19.70° (c 1, DMF).

IR (KBr): 3306, 1690, 1647, 1533, 1515, 1453, 1289, 1244, 1052, 698 and 547 $cm^{-1}$.

Anal. Calcd. for $C_{27}H_{30}N_4O_5S$: C, 62.05; H, 5.78; N, 10.71; S, 6.13

Found: C, 61.87; H, 5.86; N, 10.75; S, 6.31

With the ethyl ester (6b) the reaction is much slower and gave only a 49% yield of 9, under the same conditions.

N-Carbobenzoxytyrosyl-S-Benzylcysteinylglycine Ethyl Ester (10).—The above hydrazide (8) (11.6 g, 0.02 mole), 300 ml acetic acid, 200 ml of 1.65 N HCl in methanol and 1100 ml water were placed into a 2000 ml Erlenmeyer flask. The mixture was stirred, cooled to 0°, and a solution of 25 g of sodium nitrite in 50 ml water was added dropwise. Stirring and cooling were continued for an additional 30 min and the cold mixture was extracted with ethyl acetate (5 x 400 ml). To this extract of 9 was added 5.02 g (0.04 mole) of glycine ethyl ester. The reaction mixture was stirred at room temperature for about 2 hr, dried over anhydrous sodium sulfate, filtered by gravity, and the solvent distilled off under reduced pressure. The solid residue was dried at 56°/0.2 mm Hg, yielding 12.1 g of a light yellowish solid, mp. 175-178°, softening about 170°. This material was slightly soluble in boiling alcohols, ether, and ethyl acetate (in each case about 0.5 g/100 ml at their boiling points). The product was suspended in 200 ml methyl acetate, stirred and boiled for 10 min and filtered hot. The residue was dried, as above, yielding 4.3 g of the unchanged starting material. The filtrate was collected and dried at 56°/0.2 mm Hg., whereupon 0.35 g of yellowish, crystalline material, mp. 177-179°, was obtained. Slow evaporation of about 150 ml of the solvent, in an open 250 ml Erlenmeyer flask at 25°, gave an additional 5.15 g (47%) of the same material was obtained.

IR (KBr): 3305, 3082, 3030, 2954, 2926, 1690, 1660, 1516, 1454, 1261, 1226, 1048, and 699 $cm^{-1}$.

Anal. Calcd. for $C_{30}H_{33}N_3O_7S$: C, 62.16; H, 5.73; N, 7.24; S, 5.53

Found: C, 61.98; H, 5.65; N, 7.15; S, 5.47

S-Benzyl-L-cysteine Ethyl Ester Nitrate (11).—S-Benzyl-L-cysteine ethyl ester hydrochloride (3b) (6.99 g, 0.025 mole), 400 ml acetone and 4.25 g (0.025 mole) of silver nitrate were placed into a 500 ml Parr glass bottle. Air was displaced from the bottle by nitrogen and the flask was closed. The mixture was magnetically stirred for 15 min at room temperature, then for 30 min at 50°, cooled to room temperature; suspended solid material was removed by suction filtration through a medium porosity sintered glass funnel. The filtrate was concentrated by distilling off the acetone